Title

PATH-05. IMPLEMENTATION OF A TARGETED NEXT-GENERATION SEQUENCING PANEL FOR THE DIAGNOSIS AND PRECISION MEDICINE TREATMENT OF ADULT PATIENTS WITH WHO GRADE IV DIFFUSE GLIOMAS

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MOLECULAR PATHOLOGY AND CLASSIFICATION - ADULT AND PEDIATRIC

PATH-01. DEVELOPMENT OF A SENSITIVE MULTIPLEX ASSAY FOR DETECTION OF MUTATIONS IN IDH1 AND IDH2

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Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are the most frequently mutated metabolic genes in human cancer. They encode cytosolic and mitochondrial enzymes that catalyze the conversion of isocitrate to a-ketoglutarate (αKG), a key component in metabolic and cellular pathways including the Krebs cycle. All located within exon 4, IDH1 and IDH2 mutations are found in multiple types of human cancer including, but not limited to, acute myeloid leukemia and gliomas. IDH mutations occur in the vast majority of WHO grade II/III gliomas and secondary GBMs. Here we describe a sensitive and robust single base extension assay to detect mutations affecting amino acids 100, 105, and 132 of IDH1, and amino acids 140 and 172 of IDH2 in human clinical specimens. Accuracy studies using FFPE, blood, bone marrow, and synthetic controls showed 100% concordance in mutant identification, confirmed using orthogonal methods. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) were 100%. The assay can detect reliably the presence of 5% mutation in a wild-type background with input as low as 0.25 ng DNA (FFPE). Glioma FFPE stored at 15–30°C were found to be stable for 90 days. The IDH1/IDH2 assay has been offered as a clinical test based on its performance characteristics. In a set of 289 clinical specimens including glioma and AML, results were obtained in >98%. Consistent with other published findings, the majority of mutations in glioma affected R132 of IDH1, with other mutations less frequently identified. This IDH assay has high sensitivity, can reliably detect mutations in FFPE samples, and can be implemented as part of routine clinical practice.

PATH-02. ASSOCIATION OF IDH1 MUTATION WITH HISTOLOGICAL TYPE IN INDONESIAN GLIOMA

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Glioma is the most common primary central nervous system (CNS) tumor in adults. One of molecular biomarkers of significant interest for glioma is isocitrate dehydrogenase (*IDH*) mutation. IDH1 C.395G>A (R132H) mutation are reported to occur in 55–80% of grade II and III oligodendroglioma and astrocytomas. IDH mutations have an important role in many aspects of glioma, including glioma genesis, patients prognosis, and development of therapeutic strategies. However, information on IDH mutations in gliomas is not yet available in Indonesian population. Seventy-four glioma patients in a reference hospital in Yogyakarta, Indonesia who underwent surgery were recruited. Glioma tissues in the form of paraffin tissue blocks or fresh samples were sliced for hematoxylin eosin staining and immunohistochemical examination. Genomic DNA was extracted from the samples and IDH1 mutation status was analyzed by PCR and nucleotide sequencing. IDH1 C.395G>A (R132H) mutations were detected in 16 (21.6%) of the samples. This mutation rate is lower than the rate previously reported in Asian population. This study also found that 17.5% of astrocytic type of gliomas harboring this mutation compared to 45.45 % in other tumor types. This difference is statistically significant (p=0.037). In conclusion, IDH 1 mutation is found less frequently in Indonesian glioma, and is associated with the histological subtypes.

PATH-03. PROGNOSTIC IMPORTANCE OF TUMOR GRADE IN THE POST-GENOMIC ERA

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INTRODUCTION: In the post-genomic era of glioma biology, an emerging paradigm is that the molecular and genomic features of gliomas may be more important than tumor grade in determining prognosis. Here, we analyze The Cancer Genome Atlas (TCGA) to assess whether isocitrate dehydrogenase mutation (IDHm) and mutated methylguanine methyltransferase (mMGMT) are better prognostic indicators than tumor grade. METHODS: We identified 1,115 astrocytic gliomas of all grades. We assessed survival using univariate and multivariate Cox proportional hazards models. Multivariate models were adjusted for tumor grade, age, Karnofsky's Performance Score (KPS), mMGMT, and IDHm. RESULTS: Pearson's correlation analysis indicated significant pairwise associations between mMGMT and age (r = -0.22), KPS (r = 0.14), and tumor grade (r = -0.32) (all p< 0.05). Similarly, there were significant pairwise associations between IDHm and age (r = -0.60), KPS (r = 0.34), and tumor grade (r = -0.70) (all p< 0.05). Multivariate analysis showed that age, KPS, tumor grade, mMGMT, and IDHm independently contributed to survival prognosis. For mMGMT tumors, the median survival for grade II, III, and IV tumors was 17.10, 12.70, and 8.55 months; and for MGMT unmethylated tumors, was 13.25, 13.75, and 9.30 months, respectively. For IDHm tumors, two distinct survival distributions were observed for each tumor grade. The first distribution with survival < 60 months, exhibited median survival for grades II, III, and IV patients of 14.3, 12.5, and 16.6 months, while patients surviving >= 60 months demonstrated median survivals of 88.10, 75.15, and 91.10 months, respectively. These survival distributions in IDHm survival did not significantly differ. Wild type IDH tumors fell into a single distribution, with median survival of 7.40, 10.40, and 9.70 months for grade II, III, and IV tumors, respectively. Similar survival patterns were observed in the CGGA. CONCLUSION: Survival prognostication requires synthesis of molecular features of tumors with patient characteristics and tumor grade. For IDHm gliomas, however, tumor grade is a pertinent prognostic factor.

PATH-04. MDM2/4 AMPLIFICATION AND RISK OF HYPERPROGRESSION IN HIGH-GRADE GLIOMAS TREATED WITH CHECKPOINT INHIBITORS

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Checkpoint inhibitors are revolutionizing cancer treatment. However, there are recent reports of systemic cancer patients treated with checkpoint inhibitors with associated hyperprogressive disease (HPD), a ≥2-fold increase in tumor growth rate at first post-treatment imaging, and worse outcomes. Some reports have suggested that MDM 2/4 amplification in advanced cancer may correlate with higher risk for HPD. *MDM2/4* amplifications are relatively common and are reported in up to 20% of glioblastomas. We performed a retrospective review to assess the association between *MDM2/4* amplification and HPD in patients with high grade gliomas (HGG) treated with immune checkpoint inhibitors. Of 102 patients with HGG at our institution receiving PD1 inhibitors, 13 patients were identified to have *MDM2/4* amplification. 5 were treated upfront and 8 at recurrence with PD1 inhibitors. 7/8 patients at recurrence received concurrent bevacizumab. MRIs, prior to and following initiation of checkpoint inhibitor therapy, were evaluated for evidence of HPD. 6/13 patients had radiographic progression on the first MRI after initiation of treatment. Of these, 1 met criteria for HPD in the setting of a trial with nivolumab and vorinostat. She later resumed nivolumab and avastin with significant radiographic improvement; however, continued to clinically deteriorate and died 6 weeks later. 4/6 patients had radiographic pseudo-progression with subsequent response or stabilization of disease, 1 with pathologic confirmation after re-resection. 1 continued to progress at a similar rate prior to starting immunotherapy. In this small retrospective cohort, 1 patient with *MDM2/4* amplification had evidence of HPD after starting treatment with Nivolumab. However, concurrent treatment with vorinostat limits the ability to draw conclusions. Preliminarily, it does not seem that these patients need to be excluded from checkpoint inhibitors trials. After final collection of progression and survival data, we will compare the rate of progression of MDM2/4 amplified HGG to non-amplified MDM2/4 tumors.

PATH-05. IMPLEMENTATION OF A TARGETED NEXT-GENERATION SEQUENCING PANEL FOR THE DIAGNOSIS AND PRECISION MEDICINE TREATMENT OF ADULT PATIENTS WITH WHO GRADE IV DIFFUSE GLIOMAS

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BACKGROUND: Analysis of tumors via next-generation sequencing is now routinely used in clinical practice. The UCSF500 NGS panel became available starting in June 2015. In Dec 2017, a glioblastoma precision medicine initiative started at our institution to sequence all newly-diagnosed WHO grade IV diffuse gliomas. We review our experience over a 3-year

period. METHODS: The UCSF500 Cancer Panel assesses approximately 500 cancer-associated genes for mutations, copy number alterations, and structural rearrangements, including fusions. The test can be run on tumor DNA alone or compared with normal DNA, allowing for discrimination of germline variants. Sequencing results are analyzed by a neuropathologist with genomics expertise (D.A.S.). Results from the 165 adult WHO grade IV diffuse glioma cases sequenced to date were analyzed, including 136 glioblastomas, IDH-wildtype; 19 glioblastomas, IDH-mutant; and 10 diffuse midline gliomas, H3 K27M-mutant. RESULTS: Among the 136 IDH-wildtype glioblastomas, the most common alterations were in TERT, EGFR, CDKN2A, PTEN, NF1, TP53, PIK3R1, PDGFRA, CDK4, MDM2, LZTR1, and STAG2. Among the 19 IDH-mutant glioblastomas, the most common additional alterations were in TP53, ATRX, CDKN2A, and PDGFRA. Paired germline sequencing was performed on 71 patients, ten of which were found to harbor a germline mutation associated with increased cancer risk, including the CHEK2, MSH2, and NF1 genes. Somatic hypermutation was present in nine cases, four at initial resection and five at recurrence with a temozolomide-associated mutational signature. Among the four treatment-naïve glioblastomas with hypermutation, two were Lynch syndrome-associated in patients with damaging germline mutations in MSH2, and two were sporadic tumors that harbored somatic mutations in mismatch repair genes. CONCLUSIONS: Genomic profiling in adult glioblastoma patients results in identification of potentially actionable genetic alterations and also previously unknown germline mutations associated with increased cancer risk. A subset of glioblastomas (approximately 5%) harbor somatic hypermutation, indicating potential utility of immune checkpoint inhibition.

PATH-06. QUANTITATIVE ANALYSIS OF MGMT PROMOTER METHYLATION AND ITS PROGNOSTIC VALUE IN GLIOBLASTOMA MULTIFORME (GBM) PATIENTS TREATED WITH ALKYLATING CHEMOTHERAPY- PRELIMINARY REPORT

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OBJECTIVE: To correlate the percentage of MGMT methylation with progression-free survival (PFS) and overall survival (OS) in GBM patients receiving alkylating chemotherapy. BACKGROUND: MGMT promoter methylation is a known favorable factor for patients with GBM to have better response to the treatment with alkylating chemotherapy and better survival outcome. However, in daily practice, patients with very high percentage of MGMT methylation sometimes were observed to have a shorter survival period. This study is to investigate if the strength of the positivity is correlated to the PFS and OS in GBM patients receiving alkylating chemotherapy. METHODS and PATIENTS: Quantitative MGMT methylation measurement was performed. 5% was defined as positive methylation. Seventeen patients with a diagnosis of GBM and methylated MGMT were reviewed retrospectively. Patients were placed into 3 categories based on their MGMT methylation percentages: 5–33%, 34–66%, and 67–100%. The average PFS and OS were calculated for each category. RESULTS: The 6 patients in the 5–33% methylation category had an average PFS of 14.8 months (range 9 to 32) and OS of 27.2 months (range 10 to 42). The 8 patients in the 34–66% methylation category had an average PFS of 23.9 months (range 0 to 73) and OS of 28.1 months (range 1 to 82). The 3 patients in the 67–100% methylation category had an average PFS of 9.6 months (range 2 to 21) and censored OS of 14.7 months (range 2 to 35) as 2 of the 3 are alive. CONCLUSION: Our sample size is too small to provide conclusions. Comparing the first two methylation categories, the extent of MGMT methylation appears positively correlates with PFS (14.8 versus 23.9 months) but not OS of patients (27.2 versus 28.1 months). Data from additional 15 MGMT methylated patients after follow-ups will be added for analysis.

PATH-07. PRONEURAL GLIOMAS ARE ASSOCIATED WITH POOR SURVIVAL AND MORE LIKELY LOCATED IN PROXIMITY TO THE SUB-VENTRICULAR ZONE

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INTRODUCTION: The Cancer Genome Atlas (TCGA) revealed five sub-classes of astrocytic gliomas; four sub-classes defined by RNA expression (proneural, neural, mesenchymal, and classical), and one by isocitrate dehydrogenase mutation (IDHm). These studies demonstrated prognostic differences only with IDH mutation. Using additional patient and clinical characteristics, we determine if there is a difference in survival between the non-IDH mutated molecular sybtypes of GBM, while accounting for patient age, KPS, or tumor grade. METHODS: We identified 1,073 patients with astrocytomas of all grades from TCGA, excluding IDHm tumors to examine the potential association between RNA expression-based subtype classifications without IDHm as a confounder. We assessed survival using univariate and multivariate Cox proportional hazards analyses adjusted for age, KPS, and tumor grade. We also used The Cancer Imaging Archive (TCIA) to examine the relationship between molecular subtype and propensity for neuroanatomic location of glioblastomas (GBM). RESULTS: Univariate analyses indicated improved survival with increasing KPS (HR = 0.961, p< 0.001), and worse survival with increasing age (HR = 1.054, p< 0.001) and increasing grade (HR = 3.319, p = 0.004 for grade 3; HR = 11.432, p< 0.001 for GBM; relative to grade 2). While no survival association was observed with regards to the RNA-based subtype classification in univariate analysis, in a multivariate analysis that included age, KPS, tumor grade, and RNA-based subtype classification, proneural glioblastomas are associated with worse survival (HR = 1.524, p = 0.012) relative to the non-proneural glioblastomas. Additionally, analysis of TCIA demonstrated that proneural glioblastomas were more likely to be located near the sub-ventricular zone (SVZ, p< 0.05). CONCLUSION: Our findings suggest that RNA expression-based subtype classification has prognostic utility, and proneural subtype of astrocytoma is associated with worse survival. This subtype was more likely to be located near the SVZ, suggesting potential mechanistic insights for this survival association.

PATH-08. THE IVY GLIOBLASTOMA PATIENT ATLAS - A NOVEL CLINICAL AND RADIO-GENOMICS RESOURCE FOR EARLY PHASE CLINICAL TRIAL DESIGN AND INTERPRETATION

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Newly diagnosed GBM represents a population of increased focus in early phase clinical trials. However, a key limitation of current genomic databases of GBM, such as TCGA, is that patient populations eligible for inclusion in these databases exhibit inherent biases and exhibit limitations on the quality of clinical and imaging data available for integration with genomics. To address these limitations and to better represent the genomics of patient populations commonly enrolled to early phase clinical trials, we prospectively consented and enrolled GBM patients to the Ivy Foundation Glioblastoma Patient Atlas Project. A total of 1591 patients from 7 participating sites of the Ben and Catherine Ivy Foundation Consortium for Early Phase Clinical Trials were consented to the project and clinical data was entered into a centrally managed clinical trials database. Overall 658 subjects had pre- and post-surgical imaging centrally reviewed and recorded and 387 subjects had sufficient tissue for completion of targeted exome sequencing of approximately 500 cancer causing genes (Oncopanel or Impact). More than 308 subjects had a complete set of genomics, imaging, and clinical data, including TMZ/RT use, KPS, progression, and steroid use. Histopathological features, MGMT, and IDH mutation status were also annotated. Of the subjects with full clinical data, 171 had expired by the time of last analysis of the cohort. Genomic and clinical characteristics unique to the early phase clinical trial population compared to TCGA and other cohorts of GBM were identified and radio-genomic and other advanced population-based analyses were performed. All clinical, genomic and imaging data are being utilized to create an Ivy cBio Portal for sharing of this rich dataset within the neurooncology community.

PATH-09. CLINICAL CHARACTERISTICS OF ADULTS WITH H3 K27M-MUTANT GLIOMAS AT UCSF

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